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(54) Title: USE OF PHARMACEUTICAL AGENTS FOR RESTORING, ALLEVIATION, OR TREATMENT OF IMMUNODEFICIENCY, INCLUDING THE ALLEVIATION OR TREATMENT OF THE IMMUNE DYSFUNCTION RELATED TO INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUSES (HIV) OR RELATED VIRUSES

(57) Abstract

Use of an agent which interacts with 5HT receptors as defined herein for the preparation of a medicament for restoring, alleviation, or treatment of immunodeficiency, which can be related to major surgical procedures, blood transfusions, cancer or virus infections with Epstein Barr virus (EBV), Cytomegalo virus (CMV), measles virus, varicella virus or to infection with Human Immunodeficiency Viruses (HIV) or related viruses, e.g. as seen in pre-AIDS and AIDS. The interaction may be via an immune cell receptor, e.g. present on T cells, said receptor being structurally or functionally related to the 5HT receptors or subtypes thereof present on cells in the nervous system.

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Use of pharmaceutical agents for restoring, alleviation, or treatment of immuno deficiency, including the alleviation or treatment of the immune dysfunction related to infection with Human Immunodeficiency Viruses (HIV) or related viruses.

TECHNICAL FIELD

This invention relates to the use of an agent which interacts with 5HT receptors as defined below for increasing the proliferative capacity of immune cells from individuals suffering from one or more of the above-mentioned dysfunctions. In particular the invention relates to an agent for increasing the CD4 T cell count in such individuals. The agent is also for the preparation of a medicament for restoring, alleviation or treatment of immuno deficiency, including the alleviation or treatment of the immune dysfunction related to infection with Human Immunodeficiency Viruses (HIV) or related viruses. In particular, the invention relates to the immune dysfunction seen in pre-AIDS and AIDS. The medicaments are to be used in human or veterinary medicine. The invention also relates to a method for increasing the proliferative capacity of immune cells from individuals suffering from one or more of the above-mentioned dysfunctions.

The actions of the agents are considered to be related to a stimulation of receptor sites of cells of the immune system. Said stimulation results in the maintenance and/or reestablishment of a balanced function of the immune cells affected by a virus, virus components or other antigens. Thus, the present invention provides means for the prevention and/or restoration of the impaired immune function observed in a number of pathological pictures. Decreased immune function follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions.

In particular, the present invention provides means for the prevention and/or restoration of the impaired immune function - observed in infection with Human Immune deficiency Virus (HIV), pre-AIDS, and AIDS, infection with Cytomegalo virus (CMV), Epstein Barr virus (EBV), measles virus and varicella virus all associated with cellular immune deficiency. Said means enables the subject treated to better cope with infections and/or avoid development of various forms of neoplasia.

BACKGROUND

Decreased immune function follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions. Besides Human Immune deficiency Virus (HIV), infection with Cytomegalo virus (CMV), Epstein Barr virus (EBV) and varicella virus are all associated with cellular immune deficiency. A clinical sign of this is the occasional outbreak of herpes zoster in otherwise healthy individuals following those infections. Pneumonia caused by bacteria is also common following these infections and indicates a decreased immune function. Major surgical procedures and blood transfusion are also associated with a high frequency of infections. After blood transfusions, the immune deficiency seems to be induced by the content of donor leukocytes in the blood unit. The importance of the immune deficiency induced by major surgical procedures and bloodtransfusions is accentuated by the association with more frequent recidives of cancer after these procedures.

Cancer forms which are related to immune deficiency are in particular colon cancer, but also pancreas cancer and stomach cancers and various lymphoma in particular non-Hodgkins lymphom.

A number of retroviruses induces immune deficiencies in the subject infected by viruses, in particular the Human Immunodeficiency Virus (HIV) which causes AIDS characterized by severe cellular immune defects.

Over the last 15 years the search for pharmaceutical agents being effective in the treatment of HIV infection/AIDS has been intensive, and yet today there is no known effective cure for retrovirus infection.

Treatment with immunomodulators, e.g. interleukins, has not been successful.

Also, medicaments directed to interfere with virus replication, e.g. azidothymidine (AZT) described in DE patent 3 608 606, have not yet proven effective in restoring the immune capacity of the treated subject.

In WO 93/19766 medicaments for the treatment of functional deficiencies due to HIV infection are described. Examples of such medicaments inhibiting the cAMP/PKA pathway are the Chinese herb extract "dan-shen", antagonists of adenylate cyclase, antibodies immunologically reactive with adenylate cyclase, dideoxyadenosine, and polyadenylates.

In spite of the many attempts to find effective agents against HIV infections, still today the most common therapy regimens are directed to merely alleviating symptoms and treating secondary infections by means of specific antimicrobial agents, and in special cases treating neoplasia by means of chemotherapy.

PURPOSE OF THE INVENTION

Very often newly discovered pharmaceuticals which are found to be very effective in destroying certain microorganisms or certain cells, are also found to have severe

toxic side effects - in many cases so severe that the use in clinical practice is impossible.

The use of agents according to the present invention should enable the employment of substances which - like all medicaments - have certain side effects - but which nevertheless - when administered in dosages carefully adapted to the individual subject - should provide for an increase in the proliferative capacity of immune cells, and an increase in the CD4 T cell count in such individuals, and prevention or alleviation of immune dysfunction without causing side effects so devastating that administering to humans or animals is dissuaded.

Decreased immune function follows diseases such as infections and cancer (ref. 1), as well as major surgical procedures (ref. 2) and blood transfusions (ref. 1,2,3,4).

Besides Human Immune deficiency Virus (HIV), infection with Cytomegalo virus (CMV), Epstein Barr virus (EBV), measles virus and varicella virus all associated with cellular immune deficiency.

Further, the use of agents according to the present invention is related to the fact that HIV infected patients do not primarily suffer from the HIV infection itself, but from the impact of HIV on cells of the immune system.

Very little HIV is present in the body of infected individuals. Most of the HIV is located in the lymph nodes and other lymphatic organs, and most of the T cells in the peripheral blood in HIV seropositive subjects have a decreased function as demonstrated by decreased proliferation and cytotoxicity (ref.5). The infection alone cannot explain this general functional deficiency, since it is estimated that less than 1% of the CD4 T cells in the peripheral blood is infected with HIV (ref.6,7).

All T cells, however, circulate through the lymph nodes and other lymphatic organs. T cells are, during such passage, exposed to infectious HIV and HIV proteins from degraded or defective HIV particles. Viral proteins are produced in such a high concentration that they also can be measured in serum (ref. 8). It has been demonstrated that addition of HIV proteins to normal T lymphocytes in test tubes induced a cellular deficiency in these cells resulting ln functional anergy i.e. decreased proliferation and cytotoxicity (ref. 9,10,11,12).

It has surprisingly been found that certain agents identified and registered for the use as CNS active pharmaceuticals, e.g. antimigraine agents, anxiolytica, antidepressiva, and certain neurotransmitters, when used according to the present invention are effective for the increase in proliferative capacity of immune cells as well as an increase in the CD4 T cell count and for restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions, as well as the immune dysfunction seen for virus infections associated with cellular immune deficiency when an infected individual is exposed to said agents.

Examples are the following:

- Sumatriptan is an antimigraine agent which is described in e.g. US patent 4 816 470.

- Buspirone is described as a CNS active agent in various patents:

  In US patent 3 717 634 and US patent 4 810 789 and US patent 3 717 634 as a non-benzodiazepine anxiolytic - and in WO 93/04681 for the prevention or treatment of cognitive disorders.

- Gepirone is structurally related to buspirone, and is described in US patent 4 423 049 as a non-benzodiazepine tranquillizer .

- Ipsapirone, which is also structurally related to buspirone, is described in US patent 4 818 756 as a non-benzodiazepine anxiolytic.

- Serotonin has for a number of years been considered to have effect as a neurotransmitter in the central nervous system, and the function of serotonin as such is well investigated (cf. ref. 14 and 15). Serotonin is present in large amounts in the peripheral blood bound in thrombocytes and granulocytes. The function of serotonin in these cells is unclear.

- 8-hydroxy-2-(di-N-propylamino)tetralin (DPAT) is described as a specific 5HT receptor agonist.

By restoring the proliferative capability and the cytotoxic capacity of T cells by the use according to the present invention, HIV infected individuals should be able to fight common infection and possibly also the development of neoplasia.

MAIN ASPECTS OF THE INVENTION

A main aspect of the present invention is the use of an agent which interacts with 5HT receptors as defined herein for the preparation of a medicament for restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions. The main aspect of the present invention is in particular relates to the immune dysfunction seen for virus infections associated with cellular immune deficiency.

The term "5HT receptors" as defined herein, means receptors which have been shown - or can be shown - to interact with 5HT (serotonin). Said interacting may be of the nature of an agonistic or a partially agonistic action, or an antagonistic or a partially antagonistic action. Said 5HT receptors comprise the 5HT receptors and subtypes thereof already identified in research.

The term "restoring, alleviation, or treatment" as defined herein, means providing a subject with the agent in question in an amount which is sufficient to prophylactically slow or prevent the development of an unhealthy state due to diseases such as infections and cancer, as well as major surgical procedures and blood transfusions, including the infection with Human Immunodeficiency virus or other viruses associated with cellular immune deficiency; or sufficient to alleviate the symptoms or the diseased state; or sufficient to permanently eliminate the symptoms or treat the infection itself.

The term "Human Immunodeficiency viruses or related viruses", means HIV or other retroviruses causing immune dysfunction similar to the dysfunction seen in HIV infection, pre-AIDS and AIDS. Other examples may be infections caused by HTLV1 and HTLV2, and some of the feline and bovine retroviruses.

In a particular aspect of the present invention the restoring, alleviation, or treatment of the immune dysfunction is mediated via a direct interaction between said agent and a responsive site present on some of the immune cells in the virus infected individual.

The aim is to some degree to restore the proliferative capacity and cytotoxicity of said dysfunctional cells.

The term "immune cells", as defined herein, means cells present in the blood and/or in the hemopoietic tissue of the individual which may be infected with HIV or related viruses. Methods of determining the responsiveness of said immune cells - the target cells - are described below.

Also, certain subpopulations of such responsive cells may be determined and "targeted", e.g. cells present in lymph nodes, thymus (e.g. thymocytes) and/or spleen. One particular subpopulation of lymphocytes may be responsive forms of CD4 T cells and CD8 T cells. Furthermore, in cases where HIV have induced proliferative deficiencies in certain types of hemopoietic cells, e.g. certain bone marrow cells such as stem cells at various maturation stages, the medicaments according to the present invention can be used to provide a targeted restoration of the cellular functional capabilities. One measure for this restoration is an increased CD4 T cell count and/or an increased CD4/CD8 cell ratio.

The responsive site present on some of the immune cells in the virus infected individual, may be a cellular receptor site which is structurally or functionally related to the neuronal 5HT receptors or subtypes thereof, i.e. the 5HT receptors present on cells in the nervous system.

The response obtained by the use according to the present invention may be due to various molecular actions at the site of a receptor or near to a receptor present on the immune cells. Many of said actions are considered to be initiated and/or to prevail outside the cell exhibiting the receptor, i.e. primarily at the "exterior" side of the cell membrane.

Important examples of classes of agents to be used according to the present invention are the following:

sumatriptan, 3-[2-(dimethylamino)ethyl]-n-methyl-1H-indole-5-methansulfonamide, or the class of related compounds as defined below;

buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4,5]decane-7,9-dione, or the class of related compounds as defined below;

gepirone, 4,4-dimethyl-1-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-2,6-piperidinedione, or the class of related compounds as defined below;

ipsapirone, 2-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-1,2-benzisothiazolin-3(2H)-one 1,1 dioxide, or the class of related compounds as defined below;

serotonin, 5 hydroxytryptamine (5HT), or a derivative or precursor thereof as defined below;

8-hydroxy-2-(di-N-propylamino)tetralin (DPAT) or a derivative thereof as defined below.

In one aspect of the invention, suitable members from the class considered to interact with the 5HT1 receptors are selected to be used according the present invention.

One important class of subtypes of the 5HT1 receptors is the class comprising the 5HT1d receptors. In some reports, sumatriptan and related compounds are considered to first of all interact with said 5HT1d receptors.

Another important class of subtypes of the 5HT1 receptors is the class comprising the 5HT1a receptors. In some reports, buspirone and related compounds are considered to first of all interact with said 5HT1a receptors.

Further scientific investigation of the 5HT receptors and also of additional receptor subtypes may in the future

elucidate and clarify the intermediary molecular reactions associated with the cellular 5HT receptors.

Today, the reports of the bimolecular "events" participating in the interactions with the 5HT receptors are many and confusing in that no specific chain reaction systems can be identified. Many intermediary products may be involved, and they may vary according to the various subtypes of 5HT receptors.

A further aspect of the invention is a method for increasing the proliferative capacity of immune cells of an individual comprising exposing said immune cells to an effective amount of an agent selected from the group consisting of agonists and antagonists of the 5HT receptors of said cells, said individual being infected with HIV or a related retrovirus.

Another aspect of the invention is a method for increasing the CD4 T cell count in an individual, comprising exposing said CD4 T cells to an effective amount of an agent selected from the group consisting of agonists and antagonists of the 5HT receptors of said cells, said individual being infected with HIV or a related retrovirus.

A further aspect of the invention is a method of alleviating, restoring, or treating the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions.

This further aspect of the present invention in particular relates to the immune dysfunction seen for virus infections associated with cellular immune deficiency, which method comprises administering an effective amount of an agent which interacts with 5HT receptors as defined above.

As mentioned earlier, a number of the agents suggested for use in the methods of the invention are known and used for treatment of various CNS diseases. A particular aspect of the invention therefore relates to a method of alleviating or treating individuals infected with HIV and a related retrovirus which do not also suffer from one or more CNS diseases usually treated by the agents described above.

Another main aspect of the invention is the use of a serotonin uptake inhibitor as defined below for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

In preferred embodiments of the invention said serotonin uptake inhibitor is selected from

- citalopram, 1-[3-(dimethylamino)propyl]-1(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile;

- fluvoxamine, 5-methoxy-1-(4-trifluoromethyl)phenyl)-1-pentanone O-(2-aminoethyl)oxime;

- fluoxetine (±)-N-methyl-ϒ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine;

- paroxetine, trans-(-)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4(4-fluorophenyl)piperidine;

or a precursor or a derivative of any one of said agents.

Another aspect of the invention is a method of alleviating, restoring, or treating the immune dysfunction related to the decreased or deficient immune function,

which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions, which method comprises administering to subject an agent which is a serotonin uptake inhibitor. Said serotonin inhibitor may be selected from the group mentioned just above.

Said administration may be conducted extracorporally.

DETAILED DESCRIPTION OF THE INVENTION

Not wishing to be bound by any theory of the invention, the correct use of agents according to the Invention may influence the following mechanisms:

1) T cells being primed against antigens, e.g. micro-organisms such as Candida albicans, Cytomegalo virus, etc. will - when they encounter such microorganisms become activated. The cells will undergo a clonal expansion (proliferation) and provide a large number of T cells directed against the microorganism. This "army" of T cells will perform a direct killing (cytotoxicity) of the microorganism.

2) The restoration of the proliferative capacity may lead to increased number of CD4 T cells and CD8 T cells.

3) Clonal expansion of T cells directed against HIV antigens and such killing as just mentioned may also be directed against HIV infected cells in the body and thereby possibly reduce the amount of HIV in the body.

One aspect of the invention is the use of the agent

sumatriptan, 3-[2-(dimethylamino)ethyl]-n-methyl-1H-indole-5-methansulfonamide,

or the class of related compounds as defined herein,

for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The term "sumatriptan or the class of related compounds" as defined herein", means the compounds described in US patent 4 816 470, In so far as the biochemical actions of said compounds on an individual are similar to that of sumatriptan with respect to increase of the proliferative capacity of immune cells, increase of the CD4 T cell count and/or the stimulation of receptor sites of cells of the immune system, where said stimulation results in the maintenance and/or reestablishment of a balanced function of the immune cells affected by a virus, virus components or other antigens.

The agents used according to the present invention may also be chosen from physiologically acceptable derivatives of sumatriptan formulated as depot compositions or sustained release preparations.

The compounds may be produced by a number of processes described in said US patent, which is incorporated herein by reference.

In Denmark "Imigran". (sumatriptan) is registered by Glaxo as a drug against migraine.

A further aspect of the present invention is the use of the agent

buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4,5]decane-7,9-dione,

or the class of related pharmaceutical compounds as defined herein; or gepirone and ipsapirone or the classes of related pharmaceutical compounds as defined herein,

for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The term "buspirone, or the class of related pharmaceutical compounds, as defined herein", means the compounds described in US patent 3 717 634 - in so far as the physiological actions of said compounds on an individual are similar to that of buspirone with respect to increase of the proliferative capacity of immune cells, increase of the CD4 T cell count, and the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The compounds may be produced by a number of processes described in said US patent which is incorporated herein by reference.

In Denmark "Buspar". (buspirone) is registered by Bristol-Myers Squibb as an anxiolytic.

A further aspect of the present invention is the use of the agent

gepirone, or the class of related compounds as defined herein;

or the use of the agent

ipsapirone or the class of related compounds as defined herein

for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases, such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The term "gepirone or the class of related pharmaceutical compounds as defined herein", means the compounds described in US patent 4 423 049 - in so far as the physiological actions of said compounds are similar to that of buspirone with respect increase in the proliferative response in immune cells, increase in the CD4 T cell count, and to the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The compounds may be produced by a number of processes described in said US patent which is incorporated herein by reference.

The term "ipsapirone or the class of related pharmaceutical compounds as defined herein", means the compounds

described in US patent 4 818 756 - in so far as the physiological actions of said compounds are similar to that of buspirone with respect to increase in the proliferative response in immune cells, increase in the CD4 T cell count, and the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency. The compounds may be produced by a number of processes described in said US patent which is incorporated herein by reference.

A further aspect of the invention is the use of

serotonin

or a precursor or derivative thereof

for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The term "derivative thereof" and "precursor thereof", as defined herein, means any of the physiologically acceptable compounds having related structure and equivalent function to that of serotonin.

One of the preferred serotonin derivatives to be used according to the present invention is serotonin-creatinine sulphate.

An example of a precursor for serotonin, is 5-hydroxy tryptophane.

A further aspect of the invention is the use of the agent

8-hydroxy-2-(di-N-propylamino)tetralin (8-OH DPAT) or a derivative thereof

for the preparation of a medicament for the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

The term "derivative thereof", as defined herein, means any of the physiologically acceptable compounds having similar structure and equivalent function to that of DPAT.

Also, physiologically acceptable derivatives of DPAT formulated as depot compositions or sustained release preparations are included.

As described, the present invention relates to the use of agents which interact with 5HT receptors. Said interacting has to be adapted so as to result in - or to promote - the restoration of a balanced immune function in the infected individuals.

It is conceivable that some agents capable of interacting with certain 5HT1b and/or with certain 5HT1c receptors may be selected and used according to the present invention, in so far as their physiological action contributes to the alleviation or treatment of the immune dysfunction related to infection with HIV or related viruses.

This may also apply to certain compounds considered to interact with 5HT2 or 5HT3 receptors.

Yet another overall aspect is the use of a serotonin uptake inhibitor as defined herein for the preparation of a medicament for restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions - in particular, when said infection is related to infection with HIV or other viruses associated with cellular immune deficiency.

Another aspect of the invention is an agent and method for increasing the proliferative capacity of immune cells from individuals suffering from one or more of the above immune dysfunctions.

A still further aspect is an agent and method for increasing the CD4 T cell count in such individuals.

The term "serotonin uptake inhibitor" as defined herein, means an agent which has been shown to increase the amount of 5HT at the site of or near to the site of a 5HT receptor as defined herein.

One possible action is that said inhibitors at least partially prevent serotonin from being removed from an environment close to a 5HT receptor or a subtype thereof.

Said serotonin uptake mechanism can be described as follows: After release from serotonergic neurons, much of the released serotonin is recaptured by an active reuptake mechanism. It is well known that certain inhibitors may interfere with said mechanism.

In so far as the theory of the invention that the availability of serotonin or related compounds promotes

the restoration of the dysfunctions induced by HIV or other retrovirus infections is applicable, the agents known to increase the availability of serotonin by inhibiting serotonin uptake may be considered to also promote said restoration of the immune function.

Accordingly, said agents promote the wanted physiological response by making more serotonin available at appropriate sites in the body.

Examples of such serotonin reuptake inhibitors to be used according to the present invention are the following:

- Citalopram, 1-[3-(dimethylamino)propyl]-1-(4fluorophenyl)-1,3-dihydro-5isobenzofurancarbonitrile, or a precursor or a derivative thereof.

In Denmark "Cipramil". (citalopram) is registered by Lundbeck Pharma A/S as an antidepressivum.

- Fluvoxamine, 5-methoxy-1-(4-trifluoromethyl)phenyl)-1-pentanone O-(2-aminoethyl)oxime; or a precursor or a derivative thereof.

In Denmark "Fevarin". (fluvoxamine) Solvay Duphar is registered by Ferrosan A/S as an antidepressivum.

- Fluoxetine, (±)-N-methyl-Υ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine, or a precursor or a derivative thereof.

In Denmark "Fontex". (fluoxetine) is registered by Eli Lilly Denmark A/S as an antidepressivum.

- Paroxetine, trans-(-)-3-[(1,3-benzodioxol-5-yl-oxymethyl]-4(4-fluorophenyl)piperidine; or a precursor or a derivative thereof.

In Denmark "Seroxat". (paroxetine) is registered by Novo Nordisk A/S as an antidepressivum.

A further aspect of the use according to the present invention is the use of a combination of any one of the described medicaments.

The combinations may be combinations of

one or more of the agents which interacts with 5HT receptors as defined herein;

or

one or more serotonin uptake inhibitors as defined herein;

or

one or more of the agents which interacts with 5HT receptors as defined herein - with one or more serotonin uptake inhibitors as defined herein.

Said combinations may be formulated so as to provide combined pharmaceutical effects or even synergistic effects.

Said combinations may also - or alternatively - be formulated with the purpose of trying to minimize the side effects induced by the individual medicaments.

In yet another aspect, the following regimen may be employed: Regardless of which agents are selected to be used according to the present invention, the use may further comprise the use in combination with a specific antiviral medicament.

Said combination may be formulated so as to obtain combined effects or even synergistic effects.

Said combination may also - or alternatively - be formulated so as to minimize the side effects induced by the individual medicaments.

Furthermore, the present invention provides for a method of the restoring, alleviation, or treatment of the immune dysfunction related to the decreased or deficient immune function, which follows diseases such as infections and cancer, as well as major surgical procedures and blood transfusions, which method eomprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

In a particular embodiment according to the invention the administering is conducted extracorporally in order to specifically target the action of the agent employed to the cells in question.

Thus, treatment of the cells, e.g. the lymphocytes, may also be performed using extracorporal circulation of the blood by using a technique similar to regular plasmapheresis. In this way it is possible to use a higher concentration of the active ingredient. Before returning the cells to the patient, the active ingredient is removed, e.g. by filtration or active binding to specific membranes.

Another advantage of such administration is that the total amount of medicament to be administered "internally" may be reduced as compared to systemic administration. "Surplus" of medicament may also be eliminated extracorporally - this also reducing the total amount administered to the patient.

DETERMINATION OF DOSAGE FORMS AND AMOUNTS OF THE AGENTS

An aspect of the invention is to employ tests on living cells obtained from infected individuals in order to assess the effect of the agents on cellular immunity. Whenever appropriate or necessary, the dosage forms and amount of the agents may be determined according to the procedures described herein.

Thus, the effect of immunorestorative agents on cells from virus infected individuals, in particular pre-AIDS or AIDS patients, may be determined ln the following way:

Specially adapted isolation procedures may be employed to obtain fractions comprising the selected types of cells, the reaction physiology of which is to be determined with respect to the response to various agents.

The starting material may be blood or other cell containing body fluids, and also samples containing hemopoietic tissue, e.g. obtained by biopsy/aspiration procedures. Important material is also fluids containing bone marrow cells.

Examples of cells to be examined are in particular white blood cells, leucocytes, such as lymphocytes and predecessors thereof, including blast cells and other immature cells representing various stages in the maturation process starting from stem cells.

Measurements based on blood cells or various other cells from healthy donors may be used as standard/reference values.

In a preferred procedure, the starting material is peripheral blood, and the first step is the isolation of a fraction enriched in mononuclear cells.

Typically, peripheral blood mononuclear cells containing about 70% T lymphocytes are isolated from blood. Usually the method involves a centrifugation step, such as density gradient centrifugation.

An important aspect is to perform preliminary experiments designed to determine suitable ranges of cell concentration in relation to various concentrations of agents. Such experiments provide for reference curves applicable for deciding the optimal ratios between cell amount and agent so as to ensure a suitable biochemical response.

A predetermined amount of these cells, e.g. 5 000 to 100 000 cells, such as from 25 000 to 75 000 cells, typically approximately 50 000 of these cells are added to each well in a microtitre plate. Correspondingly, said wells are designed to each contain a maximal volume of 200 µl.

In a subsequent step, a standard "activating" stimulus is added to the cultures. This stimulus may be selected from the following:

mitogenic agents e.g. poke weed mitogen (PWM) or phytohaemagglutinin (PHA); non-specific activators of most T cells; or recall antigens, i.e. antigens from microorganisms e.g. tetanus toxoid or Candida albicans.

Subsequently, a suitable culture medium containing appropriate nutrients and buffers (e.g. RPMI supplemented with 10% fetal calf serum and antibiotics) is added to attain a known total volume, e.g. 200 µl.

The microplate cultures are incubated in an incubator under suitable conditions (such as with 5% CO_2 and 100% humidity at 37°C) for e.g. 6 days.

At day 5, a labelling agent, such as ^{3}H-thymidine (for instance 1 µCi) is added to all cultures. At day 6 cultures are harvested on glass filter plates, transferred to counting vials, and a suitable developer is added (e.g. 5 ml of scintillation fluid).

The amount of label incorporated, e.g. the amount of ^{3}H-thymidine incorporated in the DNA of the dividing cells, is measured according to methods known in the art.

The cAMP concentrations ln various cells from infected individuals may also be determined during the testing of agents and during treatment in order to elucidate the possible role of cAMP as an endogenous compound useful for monitoring of various cellular functional states.

Specific cell types may be determined and quantified according to methods known in the art. E.g. CD4 T cells and CD8 T cells may be determined by means of flow cytometry. Said cell determinations may he used to identify individuals susceptible to treatment, and said cell determinations may also be one of the parameters of a system designed to monitor the effect of treatments.

The effect of the agents to be used according to the present invention is investigated by adding said agents in various concentrations in one of the following ways:

a) the agent is added to the blood or other cell containing body fluids before the step of cell isolation is performed;

b) the agent is added to the cell enriched fraction placed in the microtitre plate prior to the addition of the mitogenic stimulus (e.g. 10 - 30 minutes earlier).

Afterwards, the reaction media containing the agent to be tested is kept under suitable conditions including a

suitable temperature until the addition of stimulus. A commonly employed temperature is room temperature.

ADMINISTRATION OF THE AGENTS

General remarks:

The agents used according to the present invention should be administered in amounts effective to attain the above mentioned restoration of immune function without inducing unbalanced effects or severe side effects.

When the dosages are determined, the following parameters should be taken into account:

- the severity of the disease;

- the weight and age of the subject to be treated;

- the functional state of various systems/organs involved in the elimination of the drug in question, in particular the renal and hepatic function;

- the toxicity and potential adverse side effects of the drug in question.

- the administration route.

The dosages given below with respect to the various agents to be used according to the present invention are to be read with respect to the above general remarks.

The concentrations relate to amount of active compound. According to the invention, the agents are used to prepare medicaments containing physiologically acceptable additives known in the art.

During test periods and treatment periods careful monitoring of side effects should be performed.

Administration of sumatriptan

According to one protocol the following dosages are employed for restoration of immune function:

Administration on a daily basis in a dose of 100-300 mg, e.g. administered as the only single initial treatment which may enable determination of the degree of response.

The results obtained are used as the basis for the choice of further dosages and for the set-up of an individually adapted regimen.

A suitable protocol for a re-treatment or maintenance treatment, would be the following:

> On a daily basis: administration of tablets 100-300 mg or alternatively 6 mg given subcutaneously.

This could be obtained by the administration of "Imigran". (Glaxo).

Administration of buspirone

The maximal therapeutic non-toxic dose of buspirone is considered to be approximately 2400 mg per day.

According to one protocol the following dosages are employed for restoration of immune function:

Administration on a daily basis in a dose of 10-60 mg, e.g. six tablets of 10 mg (total 60 mg) are administered initially as the only single initial treatment which may enable determination of the degree of response.

Side effects such as nausea, dizziness, headache, anxiety, are monitored.

The medicament "Buspar"., Bristol-Myers Squibb, may be employed.

The results obtained are used as the basis for the choice of further dosages and for the set-up of an individually adapted regimen.

A suitable protocol for a re-treatment or maintenance treatment, would be the same or a somewhat higher dosaging - with regular intervals adapted individually.

Administration of serotonin:

Serotonin is preferably administered i.v., e.g. by infusion to patients admitted to hospitals, or out patients with catheters placed intravenously.

Administration of serotonin has a systemic effect when administered in a concentration of 15 nM/min/kg. If serotonin is administered in this concentration for 2-4 hours most lymphoid cells in the peripheral blood and possibly also in the tissues have been reached. This - treatment must be repeated with regular intervals, e.g. every day. Other preparations of serotonin may be employed, e.g. sustained release preparations for subcutaneous injection of depot preparations.

Administration of DPAT

8-hydroxy-2-(di-n-propylamino)tetralin. This drug is a specific agonist of the 5HT1a receptor.

The dosaging should be monitored carefully with respect to toxicity and side effects.

In some cases a suitable dosage for this drug would be in the range of 10-1000 mg, e.g. 50 mg, given daily as tablets administered orally 1-3 times daily. Alternatively parenteral administration may be employed.

Administration of serotonin uptake inhibitors

Another approach is to increase the circulating concentration of serotonin by preventing the removal of serotonin produced in the body.

Such drugs may be given as tablets on a daily basis.

Suggested regimes are:

"Cipramil"., (LUNDBECK): In the range of 20-40 mg daily.

"Fevarin"., (SOLVAY DUPHAR): In the range of 100-200 mg daily.

"Fontex"., (ELI LILLY): In the range of 20-60 mg daily.

"Seroxat"., (NOVO NORDIC): In the range of 20-50 mg daily.

DESCRIPTION OF THE DRAWINGS

FIG. 1

Fig. 1 illustrates the effect of Imigran (sumatriptan) on lymphocytes isolated from blood from HIV seropositive subjects.

In this assay, peripheral blood mononuclear cells were incubated in wells of microtitre plates. Imigran was added in 8 different ten fold dilutions starting with 10 µg/ml. After 10 minutes the activator poke weed mitogen (PWM) was added.

The measurements are expressed in "cpm" (counts per minute) indicating the amount of labelled DNA incorporated into the dividing cells. The results are given as medians of triplicate determinations.

Control cultures:

The first column in the diagram represents cultures to which no agents had been added. The second and third

column represents the control cultures to which Imigran - but not PWM - had been added in different concentrations.

Test cultures:

The fourth column represents cultures to which PWM had been added, and the fifth and sixth column represents - cultures to which both PWM and Imigran had been added.

The results show that Imigran was able to increase/restore the proliferative response to an "activating" stimulus (PWM) added to said lymphocytes. The highest enhancements were obtained with 1 µg/ml of Imigran.

FIG. 2

Fig. 2 illustrates the effect of Buspar (buspirone) on lymphocytes isolated from blood from HIV seropositive subjects.

In this assay, peripheral blood mononuclear cells were incubated in wells of microtitre plates. Buspar was added in 8 different ten fold dilutions starting with 10 µg/ml. After 10 minutes the activator poke weed mitogen (PWM) was added.

The measurements are expressed in "cpm" (counts per minute) indicating the amount of labelled DNA incorporated into the dividing cells. The results are given as medians of triplicate determinations.

Control cultures:

The first column in the diagram represents cultures to which no agents had been added. The second and third column represents the control cultures to which Buspar but not PWM - had been added in different concentrations.

Test cultures:

The fourth column represents cultures to which PWM had been added, and the fifth and sixth column represents - cultures to which both PWM and Buspar had been added.

The results show that Buspar was able to increase/restore the proliferative response to an "activating" stimulus (PWM) added to said lymphocytes. The highest enhancements were obtained with 1 g/ml of Imigran.

FIG. 3

Fig. 3 illustrates the effect of serotonin (5HT) on lymphocytes isolated from blood from HIV seropositive subjects.

In this assay, peripheral blood mononuclear cells were incubated in wells of microtitre plates. Serotonin was added in 8 different ten fold dilutions starting with 50 µg/ml. After 10 minutes the activator poke weed mitogen (PWM) was added.

The measurements are expressed in "cpm" (counts per minute) indicating the amount of labelled DNA incorporated into the dividing cells. The results are given as medians of triplicate determinations.

Control cultures:

The first column in the diagram represents cultures to which no agents had been added. The second and third column represents the control cultures to which serotonin - but not PWM - had been added in different concentrations.

Test cultures:

The fourth column represents cultures to which PWM had been added, and the fifth and sixth column represents cultures to which both PWM and serotonin had been added.

The results show that serotonin was able to increase/restore the proliferative response to an "activating" stimulus (PWM) added to said lymphocytes. The highest enhancements were obtained with 5 µg/ml of serotonin.

FIG. 4

Fig. 4 illustrates the effect of 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) on lymphocytes isolated from blood from HIV seropositive subjects.

In this assay, peripheral blood mononuclear cells were incubated in wells of microtitre plates. DPAT was added in 8 different ten fold dilutions starting with 50 µg/ml. After 10 minutes the activator poke weed mitogen (PWM) was added.

The measurements are expressed in "cpm" (counts per minute) indicating the amount of labelled DNA incorporated into the dividing cells. The results are given as medians of triplicate determinations.

Control cultures:

The first column in the diagram represents cultures to which no agents had been added. The second and third column represents the control cultures to which DPAT - but not PWM - had been added in different concentrations.

Test cultures:

The fourth column represents cultures to which PWM had been added, and the fifth and sixth column represents cultures to which both PWM and DPAT had been added.

The results show that DPAT was able to increase/restore the proliferative response to an "activating" stimulus (PWM) added to said lymphocytes. The highest enhancements were obtained with 5 ng/ml of DPAT.

EXAMPLES OF ASSAYING THE IMMUNO-RESTORATIVE EFFECTS OF THE AGENTS TO BE USED ACCORDING TO THE PRESENT INVENTION

EXAMPLE 1

Samples of peripheral whole blood were obtained from HIV infected individuals. Blood mononuclear cells containing about 70% T lymphocytes were separated from whole blood by centrifugation on "Histopac" (density gradient separation). 50 000 of these cells were added to each well in a microtitre plate for further cultivation and testing.

The test agent Imigran (sumatriptan) was added to each well in various concentrations. The employed concentrations of Imigran were 8 different ten fold dilutions starting with 10 µg/ml.

After 10 minutes the activator PWM was added. Cultures without addition of PWM but with addition of Imigran were used as controls.

Subsequently, RPMI culture medium supplemented with 10% fetal calf serum and antibiotics was added to a final volume of 200 µl. The microplate cultures were incubated in an incubator with 5% CO_2 and 100% humidity at 37°C for 6 days. At day 5, 1 µCi ^{3}H-thymidine was added to all cultures. At day 6, cultures were harvested on glass filter plates and transferred to counting vials. Scintillation fluid (5 ml) were added, and the ^{3}H-thymidine incorporated in the DNA of dividing cells was counted.

By these experiments, it was demonstrated that Imigran was able to increase/restore the proliferative response of blood lymphocytes obtained from HIV seropositive subjects.

The highest enhancements were obtained with 1 µg/ml of Imigran. The results are given as medians of triplicate determinations and expressed in cpm.

The results are shown in diagrams in Fig. 1.

EXAMPLE 2

Samples of whole blood were obtained from HIV infected individuals. Peripheral blood mononuclear cells containing about 70% T lymphocytes were separated from blood by centrifugation on "Histopac" (density gradient separation). 50 000 of these cells were added to each well in a microtitre plate for further cultivation and testing.

The test agent Buspar (buspiron) was added to each well in various concentrations. The employed concentrations of Buspar were 8 different ten fold dilutions starting with 10 µg/ml.

After 10 minutes the activator PWM was added. Cultures without addition of PWM but with addition of Buspar were used as controls.

Subsequently, RPMI culture medium supplemented with 10% fetal calf serum and antibiotics was added to a final volume of 200 ml. Then the microplate cultures were incubated, labelled, and harvested as described in Example 1. Finally the ^{3}H-thymidine incorporated in the DNA of dividing cells was determined as described in Example 1.

By these experiments, it was demonstrated that Buspar was able to increase/restore the proliferative response of blood lymphocytes obtained from HIV seropositive subjects.

The highest enhancements were obtained with 1 µg/ml of Buspar. The results are given as medians of triplicate determinations and expressed in cpm.

The results are shown in diagrams in Fig. 2.

EXAMPLE 3

Samples of whole blood were obtained from HIV infected individuals. Peripheral blood mononuclear cells containing about 70% T lymphocytes were separated from blood by centrifugation on "Histopac" (density gradient separation). 50 000 of these cells were added to each well in a microtitre plate for further cultivation and testing.

The test agent serotonin (5HT) was added to each well in various concentrations. The employed concentrations of serotonin were 8 different ten fold dilutions starting with 50 µg/ml.

After 10 minutes the activator PWM was added. Cultures without addition of PWM but with addition of serotonin were used as controls.

Subsequently, RPMI culture medium supplemented with 10% fetal calf serum and antibiotics was added to a final volume of 200 ml. Then the microplate cultures were incubated, labelled, and harvested as described in Example 1. Finally the ^{3}H-thymidine incorporated in the DNA of dividing cells was determined as described in Example 1.

By these experiments, it was demonstrated that serotonin was able to increase/restore the proliferative response of blood lymphocytes obtained from HIV seropositive subjects.

The highest enhancements were obtained with 5 µg/ml of serotonin. The results are given as medians of triplicate determinations and expressed in cpm.

The results are shown in diagrams in Fig. 3.

In the same experiment the recall antigen tetanus was used as stimulator instead of PWM. The responses measured increased in the following way:

Patient 1: from 4900 cpm to 7500

Patient 2: from 2000 cpm to 3100

Patient 3: from 300 cpm to 1100

The concentration of serotonin that resulted in increased responses to tetanus was lower and ranged between 0.5 µg/ml to 5 ng/ml.

EXAMPLE 4

Samples of whole blood were obtained from HIV infected individuals. Peripheral blood mononuclear cells containing about 70% T lymphocytes were separated from blood by centrifugation on "Histopac" (density gradient separation). 50 000 of these cells were added to each well in a microtitre plate for further cultivation and testing. The test agent 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) was added to each well in various concentrations. The employed concentrations of DPAT were 8 different ten fold dilutions starting with 50 µg/ml.

After 10 minutes the activator PWM was added. Cultures without addition of PWM but with addition of DPAT were used as controls.

Subsequently, RPMI culture medium supplemented with 10% fetal calf serum and antibiotics was added to a final volume of 200 ml. Then the microplate cultures were incubated, labelled, and harvested as described in Example 1. Finally the ^{3}H-thymidine incorporated in the DNA of dividing cells was determined as described in Example 1.

By these experiments, it was demonstrated that DPAT was able to increase/restore the proliferative response of blood lymphocytes obtained from HIV seropositive subjects.

The highest enhancements were obtained with 5 ng/ml of DPAT. The results are given as medians of triplicate determinations and expressed in cpm.

The results are shown in diagrams in Fig. 4.

A CLINICAL TRIAL EMPLOYING SEROTONIN

In a clinical experiment serotonin in the form of serotonin-creatinine sulfate is administered and prepared as described by Stone et al. (10). According to Stone et al. maximum doses are 15 nM/kg/min with infusion of 8 ml/min. This equals 0.12 nmol/kg/min (8.4 nmol/min for a 70 kg patient) (MW = 176) or 21 ng/kg/min (1.5 µg/min for a 70 kg patient). Administration for 4 hours gives a total of 101 nmol/kg (7 µmol for a 70 kg patient) or 5 µg/kg (353 mg for a 70 kg patient).

Stone et al. (1993) describe how 5HT is administrated i.v. The sole side effect was an increase in the diastolic blood pressure of a few mmHg. The procedure of administration described by Stone can be followed step by step. However, the procedures as described herein aim at continuing the infusion of serotonin over a period of 4 hours.

Serotonin is administered according to the following schedule:

a) To prevent spasms in local veins, 8 ml/min of 0.15 M (isotone) saline is given for 15 minutes i.v.

b) During the following 15 minutes, the administration of serotonin is started by mixing this to the saline i.v. drop using an electronic drop counter. The serotonin solution is 5 mM serotonin dissolved in 0.15 mM saline. The infusion is kept at 8 ml/min using electronic drop counters.

c) The start concentration of serotonin is 21 ng/kg/min and increasing over 15 minutes to max. 21 ng/kg/min. This gives a maximum infusion of 1.5 µg/min for a person of 70 kg. The only side effect at this dosage level should be an increase in blood pressure of 5 mmHg diastolic (ref. 15, Stone et al.).

d) With continuous monitoring of blood pressure, pulse, EKG and O_2 saturation, the administration of serotonin is continued for 4 hours. The blood pressure is used to monitor the administration of serotonin. With an increase of 5 mmHg diastolic, the administration is decreased so that the increase in diastolic blood pressure is kept below 5 mmHg. The patient is under constant clinical surveillance.

Judged from experiments made by Stone et al (ref. 15) in which serotonin is given to 8 volunteers in the same concentration, it is not likely that infusion of serotonin in a corresponding concentration over 4 hours should give any safety problems as long as the patients are under constant surveillance and the dose is regulated to counter an increase in blood pressure. The blood serotonin concentration is measured before the start of the study and after 1, 4, and 20 hours.

The following side effects are monitored carefully: Nausea, dizziness, headache, anxiety. And for very high concentrations: Increases or decreases in blood pressure and bradycardia as well as gastrointestinal symptoms in the form of diarrhoea.

(Reference to concentrations of serotonin occurring in patients with carcinoid tumours (in the midgut): The serotonin concentrations have been measured to 256 ± 56 ng/ml, whereas the concentration in normal donors is 46 ± 10 ng/ml, i.e. a five time increase in concentration. Free

serotonin in plasma is removed in the liver and in the lungs, e.g. after i.v. administration 90% of serotonin is removed during the first pass through the lungs. 90% of administered serotonin is immediately bound to plasma serotonectin.)

5 HIV seropositive subjects who's T cells respond to serotonin in vitro were selected. Infusion of serotonin creatinin sulphate over four hours as described above.

The following results were observed:

In vitro enhancement or proliferation by addition of serotonin.

In vivo a decline in proliferation after infusion of serotonin explainable as a homing to the lymphatic tissues.

Stable leukocyte and lymphocyte counts.

Conclusion:

Serotonin infusion is able to affect peripheral T cells and induce a homing. A steady state resulting in enhanced proliferative capacity of peripheral T cells was not achieved during this short experiment, but must be expected with continuous treatment. The strongest effect was achieved in individuals with high CD4 T cell counts.

SYNOPSIS OF DATA FROM THE FIRST 3 PATIENTS TREATED WITH BUSPIRON

3 HIV seropositive subjects with CD4 T cell counts above 200 were selected. All received 60 mg of Buspiron perorally as tablets in one dose.

The following results were observed:

In vitro enhancement of proliferation by addition of Buspiron.

In vivo a decline in proliferation after 60 mg of Buspiron.

Stable leukocyte and lymphocyte counts.

CD4 increase in two patients ending more than 50 CD4 cells higher after 1 week to 1 month.

Conclusion:

Buspiron is able to affect peripheral T cells and induce a homing to the lymphatic tissues. A steady state resulting in enhanced proliferative capacity of peripheral T cells was not achieved during this short experiment, but an enhancement of the immune system by continuous treatment is expected.

The obtained results appear from the drawing.

Figures:

Fig. 5: **The cell activation.** From T cell activation to cell proliferation, the cell is guided by two pathways. The activation pathway which includes the T cell receptor, inositol phospholipid metabolism (PIP2 to IP3 and DAG), calcium influx, and activation of PKC. The inhibitory pathway that balance the other, is the PKA pathway, which only is active in the presence of cAMP. cAMP is generated by the enzyme adenylate cyclase. In T lymphocytes from HIV seropositive subjects there is an increased activity of the inhibitory PKA/cAMP pathway. Inhibition of this pathway restores the immune function in T cells from HIV seropositive subjects.

Fig. 6: **The normal immune system.** In the normal immune system T cells circulate through the lymph nodes and other

lymphatic tissues into the peripheral blood and back. Only about 2% or all lymphocytes are in the peripheral blood. The rest are in the tissues. When a T lymphocyte on its passage encounters a macrophage presenting a microbial antigen (virus, fungus etc.) with the right specificity for the T cell, the cell will start to proliferate. This proliferation generates an army of effector cells that will fight the foreign microbial organism (first generating an expansion of CD4 T helper cells which in turn provide help for B cells and CD8 cytotoxic cells in a cascade). In HIV infection these final steps are blocked because T cells have decreased proliferative capacity and decreased cytotoxic capacity.

Fig. 7: **Buspiron increases the proliferative response of lymphocytes from HIV seropositive subjects in vitro.** Buspiron increases the T cells' capacity to proliferate in vitro experiments as shown above with relation to Fig. 2. By treating HIV seropositive patients with Buspiron it must be expected that the proliferative capacity of the T cells will increase, first in the tissues, because in vivo the immune reactions will not take place in the peripheral blood, and later, when a steady state is reached, an increased proliferative capacity should also be detected in the peripheral blood. Also an increased proliferative capacity may result in a higher number of newly generated CD4 T cells.

At time zero, blood was drawn from all patients. Histopaq density gradient purified peripheral blood nononuclear cells (PBMC) were added to microtitre plates in triplicates. Total volume was 200 µl of RPMI supplemented with 10% Fetal calf serum. After 5 days in culture, 2 µCi of 3H-thymidine was added to all cultures. At day 6, all cultures were harvested on glass filter mats and counted

in a beta-counter after addition of scintillation fluid. Cultures were without or with Buspiron added.

Fig. 8: Buspiron induces a homing of reactive peripheral lymphocytes to the lymphatic tissues within a few hours when given in a single dose of 60 mg perorally. The lymphocyte proliferative capacity to stimulation with PWM was measured at time 0, 1 h, 4 h, 24 h, 1 week and 1 month (data not ready yet) as described above. Because the drop in reactive lymphocytes happens within one hour, a redistribution is evident.

Fig. 9: Two or three patients seem to increase in CD4 T cells. In two patients with decreased CD4 T cell numbers an increase of more than 50 was seen after 1 week/1 month. An increase after one week or more indicates a production more than a redistribution. No changes were seen in the patient who had the highest CD4 T cell count from the beginning. Normal level is above 500 CD4 T cells per. µl.

Fig. 10: Two of three patients increase in their CD4/CD8 T cell ratio. In two patients an increase in CD4/CD8 ratio was seen after one week. Normal ratios are above 1.0.

REFERENCES

1 Houbiers JGA, Brand A, Van De Watering LMG, Hermans J, Verwey PJM, Bijnen AB, Pahlplatz P, Schattenkerk ME, Wobbes T, et al. Randomised controlled trial comparing transfusion of leucocyte-depleted or buffy-coat-depleted blood in surgery for colorectal cancer. Lancet. 1994; 344: 573-578.

2 Agarwal N, Murphy JG, Cayten CG, Stahl WM. Blood transfusion increases the risk of infection after trauma. Arch Surg. 1993; 128: 171-177.

3 Jensen LS, Andersen AJ, Christiansen PM, Hokland P, Juhl CO, Madsen G, Mortensen J, Moller-Nielsen C, Hanberg - Sorensen F, Hokland M. Postoperative infection and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. Br J Surg. 1992; 79: 513-516.

4 Edna T-H, Bjerkeset T. Association between blood transfusions and infection in injurred patients. J. Trauma. 1992; 33: 659-661.

5 Hofmann B, Jakobsen K, Odum N, Dickmeiss E, Platz P, Ryder L, Petersen C, Mathisen L, Bygbjerg I, Faber V, Svejgaard A. HIV-induced immunodeficiency: Relatively preserved PHA as opposed to decreased PWM responses may be due to possibly preserved responses via CD2/PHA pathway. J. Immunol. 1989; 142: 1874-1880.

6 Harper ME, Marselle LM, Gallo RC, Wong-Staal F. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by in situ hypridization. PNAS. 1989; 83: 772-776.

7 Schnittman SM, Greenhouse JJ, Psallidopoulos MC, Baseler M, Salzman NP, Fauci AS, Lane HC. Increasing viral burden in $CD4^+$ T cells from patients with HIV infection reflects rapidly progressive immunosuppression and clinical disease. Ann. Internal Medicine 1990; 113: 438-443.

8 Nishanian P. Huskins K.S., Stehn S., Detels R., Fahey JL. A simple method for improved assay demonstrates that HIV p24 is present as immune complexes in most sera from HIV-infected individuals. J. Infect. Dis. 1990, 162: 21.

9 Hofmann B, Nishanian P, Baldwin RL, Insixiengmay P, Nell A, Fahey JL. HIV inhibits the early steps of lymphocyte activation, including initiation of inositol phospholipid metabolism. J. Immunol. 1990; 145: 3699-3705.

10 Hofmann B, Nishanian P, Fan J, Nguyen T, Fahey JL. HIV GAG p17 impairs proliferation of normal lymphocytes in vitro. AIDS 1994; Vol. 8, No. 7; 1016-1017.

11 Hofmann B, Nishanian P, Nguyen T, Insixiengmay P, Fahey JL. HIV proteins induce the inhibitory cAMP/protein kinase A pathway in normal T lymphocytes. Proceedings of National Academy of Science, USA 1993; 90: 6676-6680.

12 Hofmann B, Nishanian P, Nguyen T, Liu M, Fahey JL. Restoration of T cell function in HIV infection by reduction of intracellular CAMP levels with adenosine analogues. AIDS 1993; 7: 659-664.

13 Louis S. Goodman and Alfred Gilman. The Pharmacological Basis of Therapeutics. Fifth edition 1975. Later editions contain only a reduced description of serotonin.

14 Stone et al. Effect of 5-hydroxytryptamin and 5-hydroxytryptophan infusion of the human cough reflex. J. Appl. Physiol. 1993; 74: 396-401

15 Paul M. Vanhoutte. Serotonin and the cardiovascular system. Raven Press, New York, 1985.

C L A I M S

1. Use of an agent which interacts with 5HT receptors as defined herein for the preparation of a medicament for restoring, alleviation, or treatment of immuno deficiency.

2. Use according to claim 1 wherein the restoring, alleviation, or treatment of immuno deficiency is related to major surgical procedures.

3. Use according to claim 1 wherein the restoring, alleviation, or treatment of immuno deficiency is related to cancer.

4. Use according to claim 1 wherein the restoring, alleviation, or treatment of immuno deficiency is related to blood transfusions.

5. Use according to claim 1 wherein the restoring, alleviation, or treatment of immuno deficiency is related to a viruses associated with cellular immune deficiency.

6. Use according to claim 5 wherein the infection of Human Immune deficiency Virus (HIV) is the cause of cellular immune deficiency.

7. Use according to claim 5 wherein the infection of Epstein Barr virus (EBV) is the cause of cellular immune deficiency.

8. Use according to claim 5 wherein the infection of Cytomegalo virus (CMV) is the cause of cellular immune deficiency.

9. Use according to claim 5 wherein the infection of measles virus is the cause of cellular immune deficiency.

10. Use according to claim 5 wherein the infection of varicella virus is the cause of cellular immune deficiency.

11. Use according to any of the preceding claims, wherein the alleviation or treatment of the immune dysfunction is mediated via a direct interaction between said agent and a responsive site present on some of the immune cells as defined herein.

12. Use according to claim 11, wherein said site is a cellular receptor which is structurally or functionally related to the 5HT receptors or subtypes thereof present on cells in the nervous system.

13. Use according to claim 11 or 12, wherein said immune cells are lymphoid cells or stem cells thereof, e.g. T cells, such as CD4 T cells and CD8 T cells.

14. Use according to any of the preceding claims, wherein the agent is sumatriptan, 3-[2-(dimethylamino)ethyl]-n-methyl-1H-indole-5-methansulfonamide, or the class of related compounds as defined herein.

15. Use according to any of claims 1-13, wherein the agent is buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4,5]decane-7,9-dione, or the class of related compounds as defined herein.

16. Use according to any of claims 1-13, wherein the agent is gepirone, 4,4-dimethyl-1-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-2,6-piperidinedione, or the class of related compounds as defined herein; or ipsapirone, 2-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-1,2-benzisothiazolin-3(2H)-one 1,1 dioxide, or the class of related compounds as defined herein.

17. Use according to any of claims 1-13, wherein the agent is serotonin, 5 hydroxytryptamine (5HT), or a derivative or precursor thereof as defined herein.

18. Use according to any of claims 1-13, wherein the agent is 8-hydroxy-2-(di-N-propylamino)tetralin (DPAT) or a derivative thereof as defined herein.

19. Use according to any of the preceding claims, wherein said medicament further comprises a specific antiviral agent.

20. A method for restoring, alleviation, or treatment of immuno deficiency related to related to major surgical procedures, which method comprises administering an effective amount of an agent which interacts with 5HT receptors as defined herein.

21. A method for restoring, alleviation, or treatment of immuno deficiency related to related to cancer, which method comprises administering an effective amount of an agent which interacts with 5HT receptors as defined herein.

22. A method for restoring, alleviation, or treatment of immuno deficiency related to related to blood transfusions, which method comprises administering an effective amount of an agent which interacts with 5HT receptors as defined herein.

23. A method for restoring, alleviation, or treatment of immuno deficiency related to a viruses infection associated with cellular immune deficiency, which method comprises administering an effective amount of an agent which interacts with 5HT receptors as defined herein.

24. A method according to claim 23 wherein the infection is cause by Human Immune deficiency Virus (HIV).

25. A method according to claim 23 wherein the infection is cause by Epstein Barr virus (EBV).

26. A method according to claim 23 wherein the infection is cause by Cytomegalo virus (CMV).

27. A method according to claim 23 wherein the infection is cause by measles virus.

28. A method according to claim 23 wherein the infection is cause by varicella virus.

29. A method of increasing the proliferative capacity of immune cells of an individual suffering from immunodeficiency as stated in any of claims 20-28 comprising exposing said immune cells to an effective amount of an agent selected from the group consisting of agonists and antagonists of the 5HT receptors of said cells.

30. A method of increasing the CD4 T cell count in an individual suffering from immunodeficiency as stated in any of claims 20-28 comprising exposing said CD4 T cells to an effective amount of an agent selected from the group consisting of agonists and antagonists of the 5HT receptors of said cells.

31. A method according to to any of claims 20-30 wherein said agent is selected from

- sumatriptan, 3-[2-(dimethylamino)ethyl]-n-methyl-1H-indole-5-methansulfonamide, or the class of related compounds as defined herein;

- buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4,5]decane-7,9-dione, or the class of related compounds as defined herein;

- gepirone, 4,4-dimethyl-1-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-2,6-piperidinedione, or the class of related compounds as defined herein;

- ipsapirone, 2-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-1,2-benzisothiazolin-3(2H)-one 1,1 dioxide, or the class of related compounds as defined herein;

- serotonin, 5 hydroxytryptamine (5HT), or a derivative or precursor thereof as defined herein;

- 8-hydroxy-2-(di-N-propylamino)tetralin (DPAT) or a derivative thereof as defined herein.

32. A method for restoring, alleviation, or treatment of immuno deficiency related to related to major surgical procedures, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

33. A method for restoring, alleviation, or treatment of immuno deficiency related to related to cancer, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

34. A method for restoring, alleviation, or treatment of immuno deficiency related to related to blood transfusions, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

35. A method for restoring, alleviation, or treatment of immuno deficiency related to related to a viruses infection associated with cellular immune deficiency, which method comprises administering an effective amount

of an agent capable of stimulating a site present on some of the immune cells as defined herein.

36. A method according to claim 35 wherein the infection is caused by Human Immune deficiency Virus (HIV).

37. A method according to claim 35 wherein the infection is caused by Epstein Barr virus (EBV).

38. A method according to claim 35 wherein the infection is caused by Cytomegalo virus (CMV).

39. A method according to claim 35 wherein the infection is caused by measles virus.

40. A method according to claim 35 wherein the infection is caused by varicella virus.

41. A method according to any of claims 20-40, being conducted extracorporally.

42. A method for restoring, alleviation, or treatment of immuno deficiency related to related to major surgical procedures, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

43. A method for restoring, alleviation, or treatment of immuno deficiency related to related to cancer, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

44. A method for restoring, alleviation, or treatment of immuno deficiency related to related to blood transfusions, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

45. A method for restoring, alleviation, or treatment of immuno deficiency related to related to a viruses infection associated with cellular immune deficiency, which method comprises administering an effective amount of an agent capable of stimulating a site present on some of the immune cells as defined herein.

46. A method according to claim 45 wherein the infection is caused by Human Immune deficiency Virus (HIV).

47. A method according to claim 45 wherein the infection is caused by Epstein Barr virus (EBV).

48. A method according to claim 45 wherein the infection is cause by Cytomegalo virus (CMV).

49. A method according to claim 45 wherein the infection is caused by measles virus.

50. A method according to claim 45 wherein the infection is caused by varicella virus.

51. Use of serotonin uptake inhibitor as defined herein for the preparation of a medicament for restoring, alleviation or treatment of the immune dysfunction related to infections with HIV viruses or related viruses.

52. Use according to claim 51, wherein said serotonin uptake inhibitor is selected from

- citalopram, 1-[3-(dimethylamino)propyl]-1(4-fluorophenyl)-1,3-dihydro-5-isobenzofuran-carbonitrile;

- fluvoxamine, 5-methoxy-1-(4-trifluoromethyl)phenyl)-1-pentanone O-(2-aminoethyl)oxime;

- fluoxetine, (±)-N-methyl-ϒ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine;

- paroxetine, trans-(-)-3-[(1,3-benzodioxol-5-yl-oxy)methyl]-4(4-fluorophenyl)piperidine;

or a precursor or a derivative of any one of said agents.

53. Use according to any one of claims 51-52, wherein said medicament further comprises a specific antiviral agent.

54. A method for restoring, alleviation, or treatment of immuno deficiency related to related to major surgical procedures, which method comprises administering an effective amount of an agent which is a serotonin uptake inhibitor.

55. A method for restoring, alleviation, or treatment of immuno deficiency related to related to cancer, which method comprises administering an effective amount of an agent which is a serotonin uptake inhibitor.

56. A method for restoring, alleviation, or treatment of immuno deficiency related to related to blood transfusions, which method comprises administering an effective amount of an agent which is a serotonin uptake inhibitor.

57. A method for restoring, alleviation, or treatment of immuno deficiency related to related to a viruses infection associated with cellular immune deficiency, which method comprises administering an effective amount of an agent which is a serotonin uptake inhibitor.

58. A method according to claim 57 wherein the infection is caused by Human Immune deficiency Virus (HIV).

59. A method according to claim 57 wherein the infection is caused by Epstein Barr virus (EBV).

60. A method according to claim 57 wherein the infection is caused by Cytomegalo virus (CMV).

61. A method according to claim 57 wherein the infection is caused by measles virus.

62. A method according to claim 57 wherein the infection is caused by varicella virus.

63. A method according to claim 54-62, wherein said serotonin uptake inhibitor is selected from

- citalopram, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile;
- fluvoxamine, 5-methoxy-1-(4-trifluoromethyl)phenyl)1-pentanone O-(2-aminoethyl)oxime;
- fluoxetine, (±)-N-methyl-γ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine;
- paroxetine, trans-(-)-3-[(1,3-benzodioxol-5-yl-oxy)methyl]-4(4-fluorophenyl)piperidine;

or a precursor or a derivative of any one of said agents.

64. A method according to claim 54-63, being conducted extracorporally.

FIG. 1

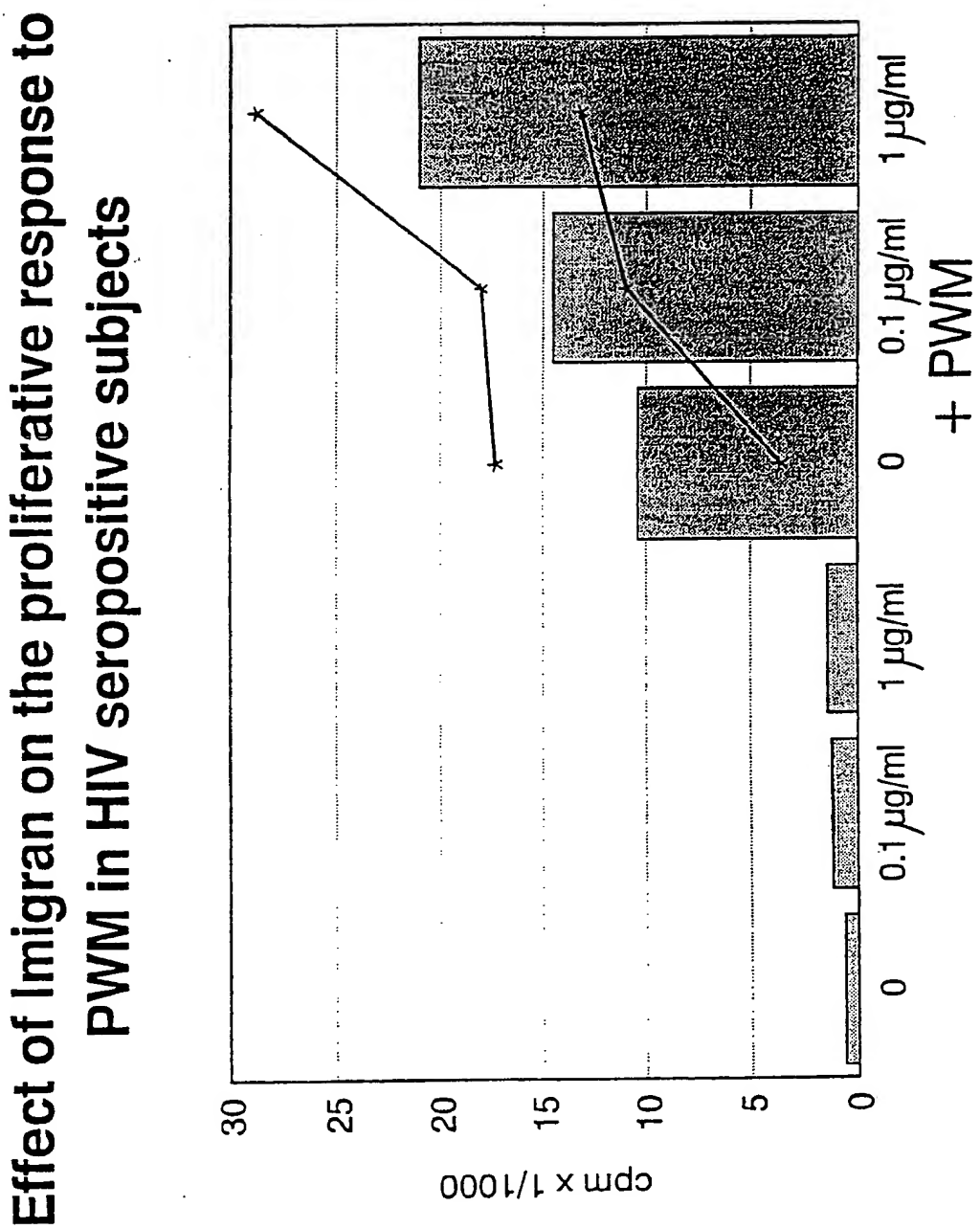

Effect of Imigran on the proliferative response to PWM in HIV seropositive subjects
cpm x 1/1000
30
25
20
15
10
5
0
0
0.1 µg/ml
1 µg/ml
0
0.1 µg/ml
1 µg/ml
+ PWM

FIG. 2

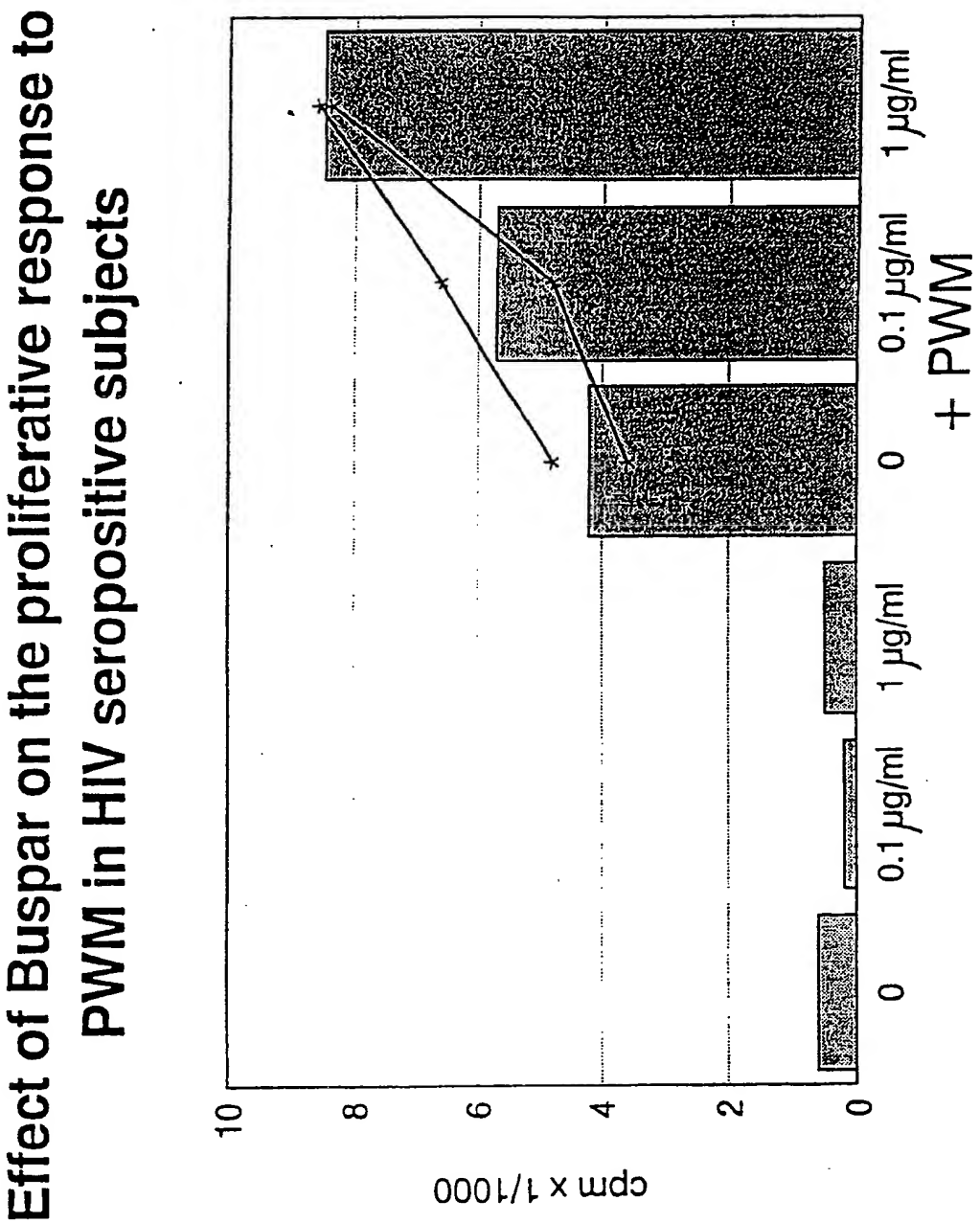

Effect of Buspar on the proliferative response to PWM in HIV seropositive subjects
cpm x 1/1000
10
8
6
4
2
0
0
0.1 µg/ml
1 µg/ml
0
0.1 µg/ml
1 µg/ml
+ PWM

FIG. 3

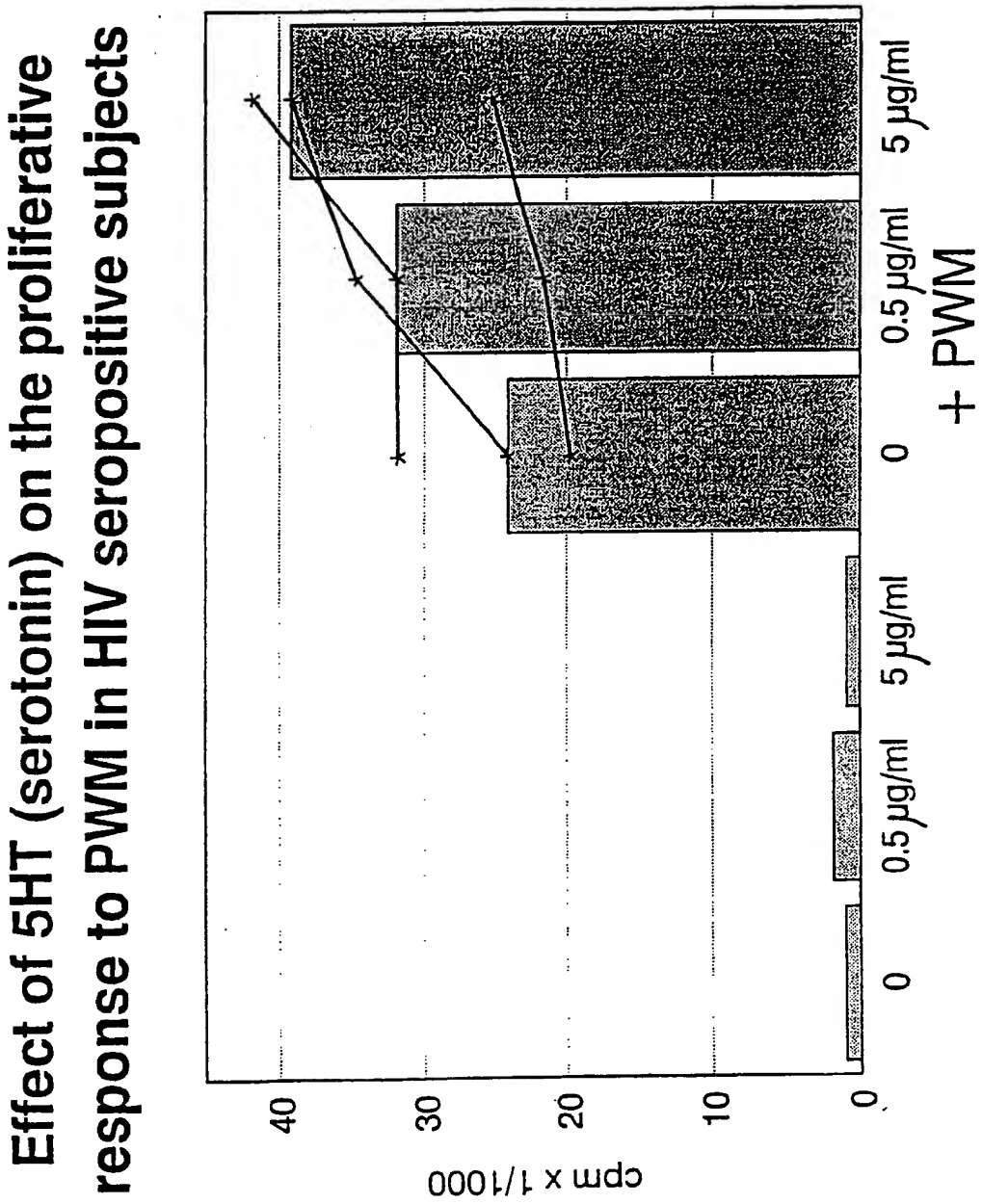

Effect of 5HT (serotonin) on the proliferative response to PWM in HIV seropositive subjects
cpm x 1/1000
0
10
20
30
40
0
0.5 µg/ml
5 µg/ml
0
0.5 µg/ml
5 µg/ml
+ PWM

FIG. 4

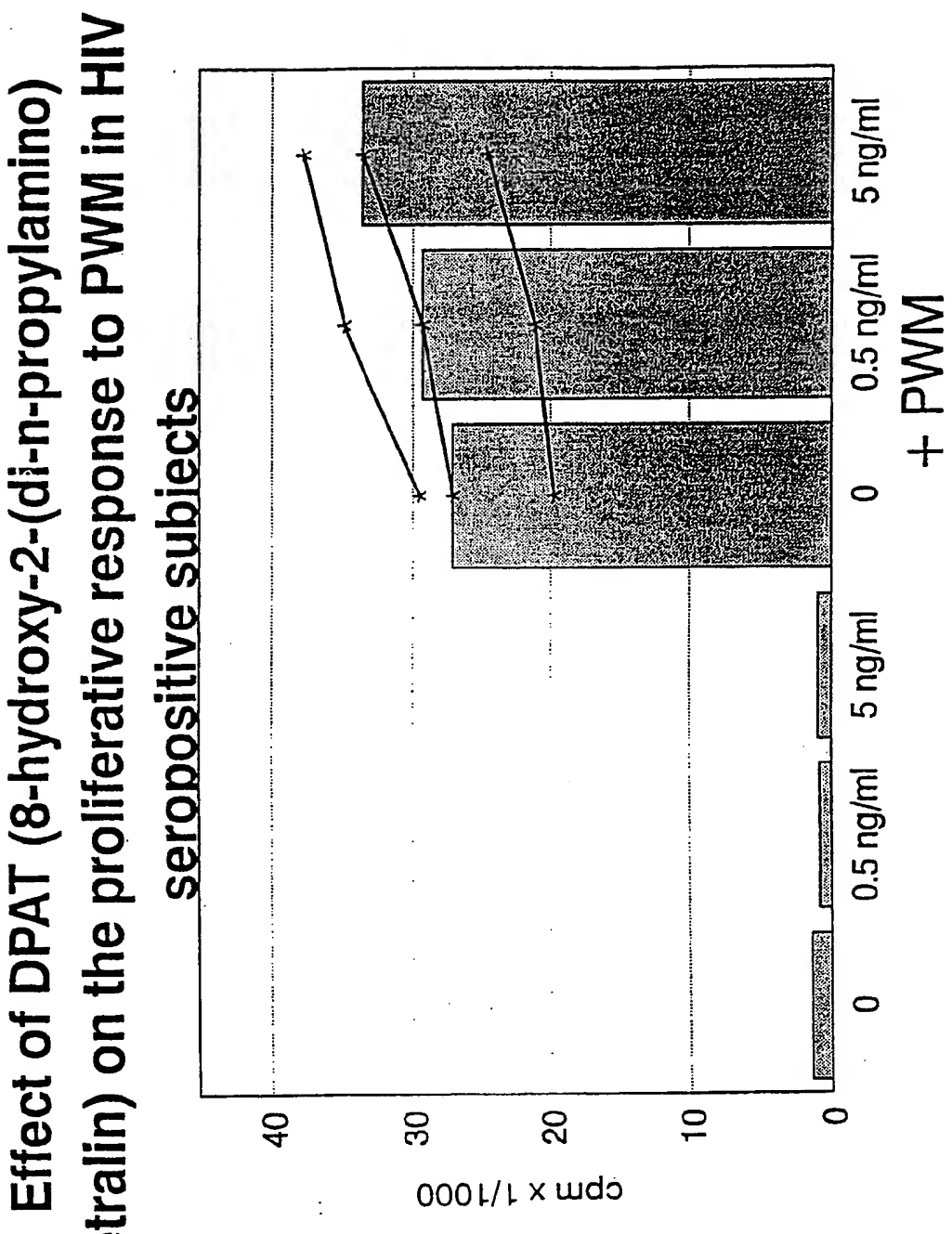

Effect of DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) on the proliferative response to PWM in HIV seropositive subjects
cpm x 1/1000
0
10
20
30
40
0
0.5 ng/ml
5 ng/ml
0
0.5 ng/ml
5 ng/ml
+ PWM

FIG. 5

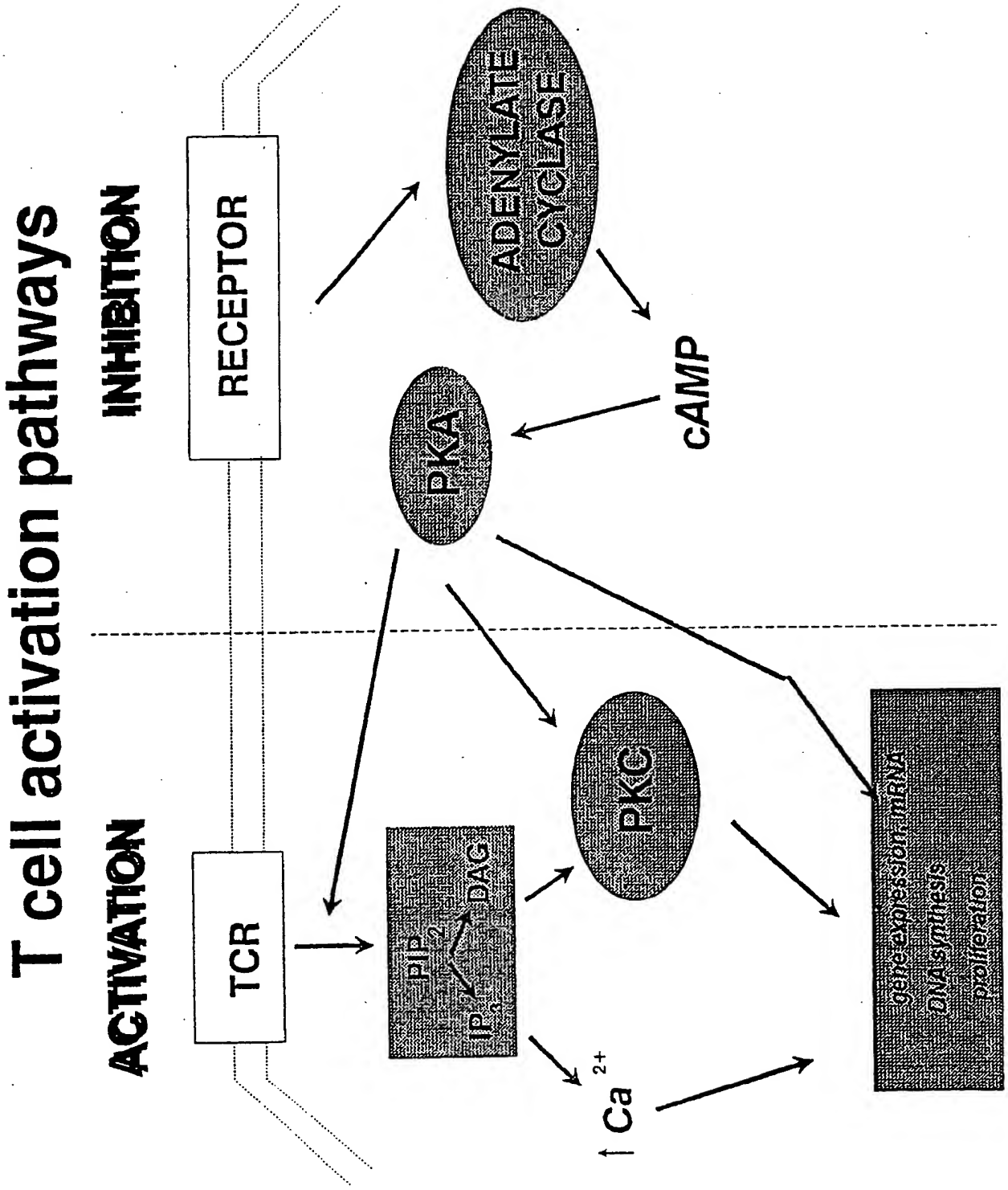

T cell activation pathways
INHIBITION
ACTIVATION
RECEPTOR
TCR
ADENYLATE CYCLASE
cAMP
PKA
PKC
PIP2
IP3
DAG
↑ Ca2+
gene expression mRNA
DNA synthesis
proliferation

FIG. 6

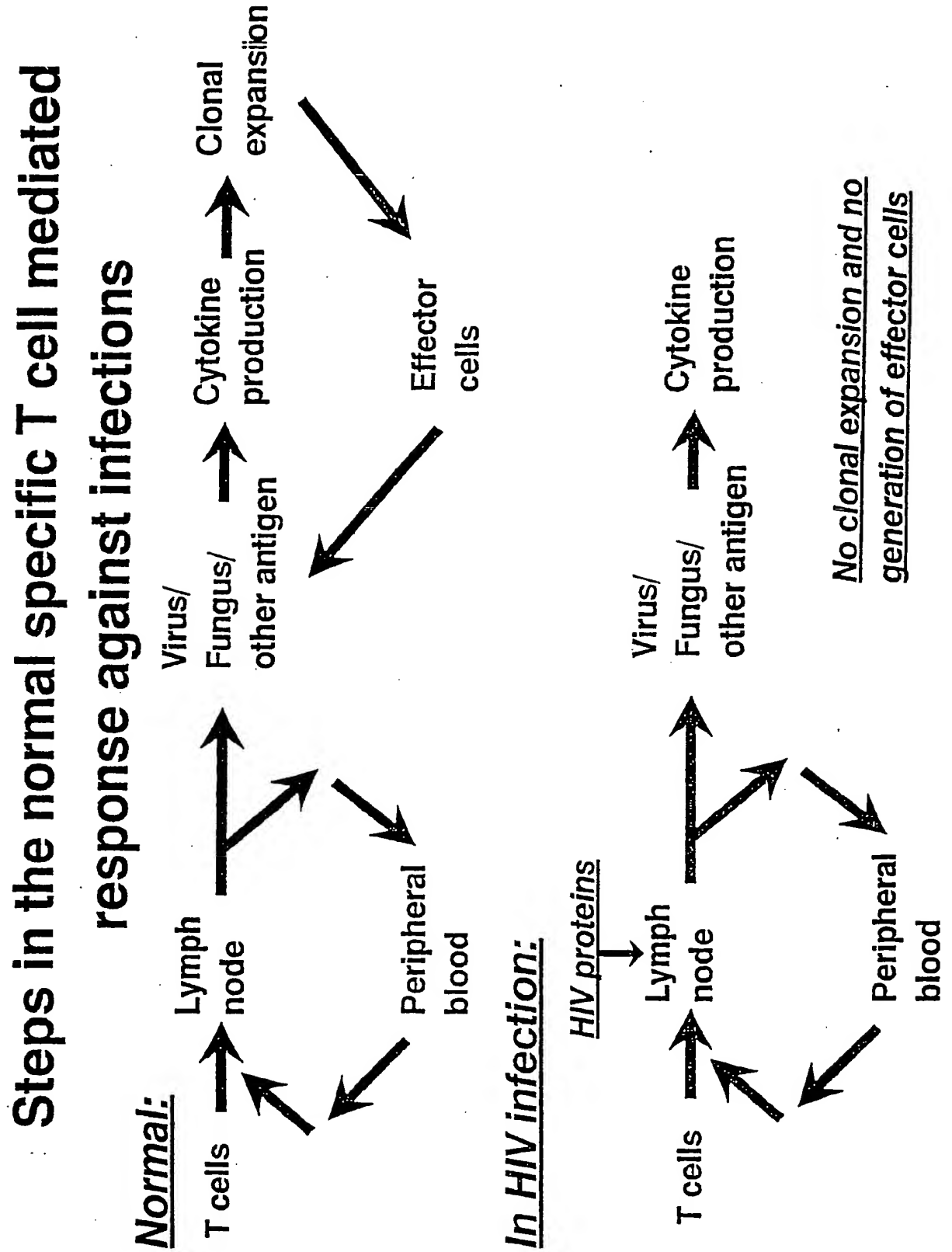

Steps in the normal specific T cell mediated response against infections
Normal:
T cells
Lymph node
Peripheral blood
Virus/ Fungus/ other antigen
Cytokine production
Clonal expansion
Effector cells
In HIV infection:
HIV proteins
T cells
Lymph node
Peripheral blood
Virus/ Fungus/ other antigen
Cytokine production
No clonal expansion and no generation of effector cells

FIG. 7

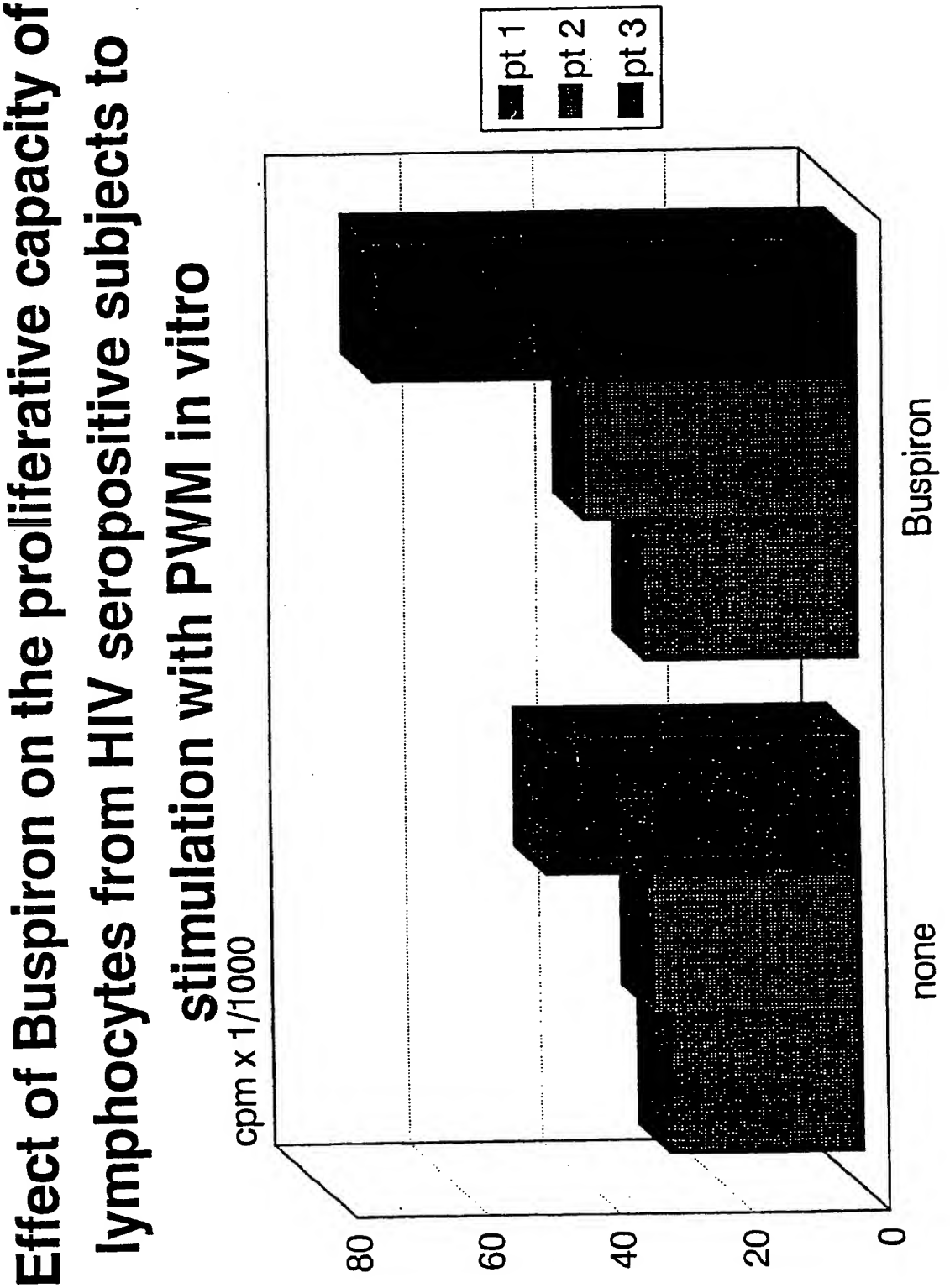

Effect of Buspiron on the proliferative capacity of lymphocytes from HIV seropositive subjects to stimulation with PWM in vitro
cpm x 1/1000
0
20
40
60
80
pt 1
pt 2
pt 3
none
Buspiron

FIG. 8

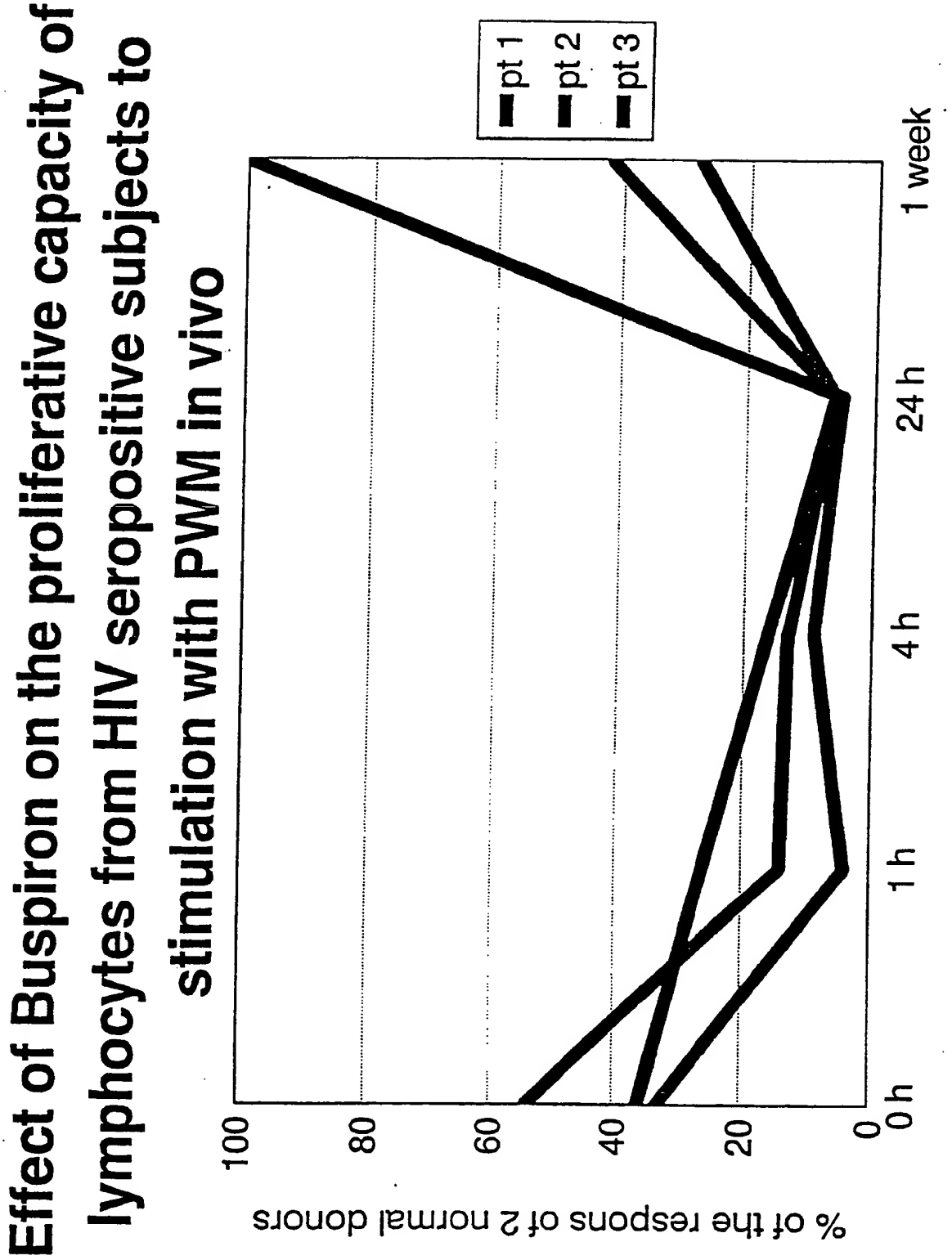

Effect of Buspiron on the proliferative capacity of lymphocytes from HIV seropositive subjects to stimulation with PWM in vivo
pt 1
pt 2
pt 3
% of the respons of 2 normal donors
100
80
60
40
20
0
0 h
1 h
4 h
24 h
1 week

FIG. 9

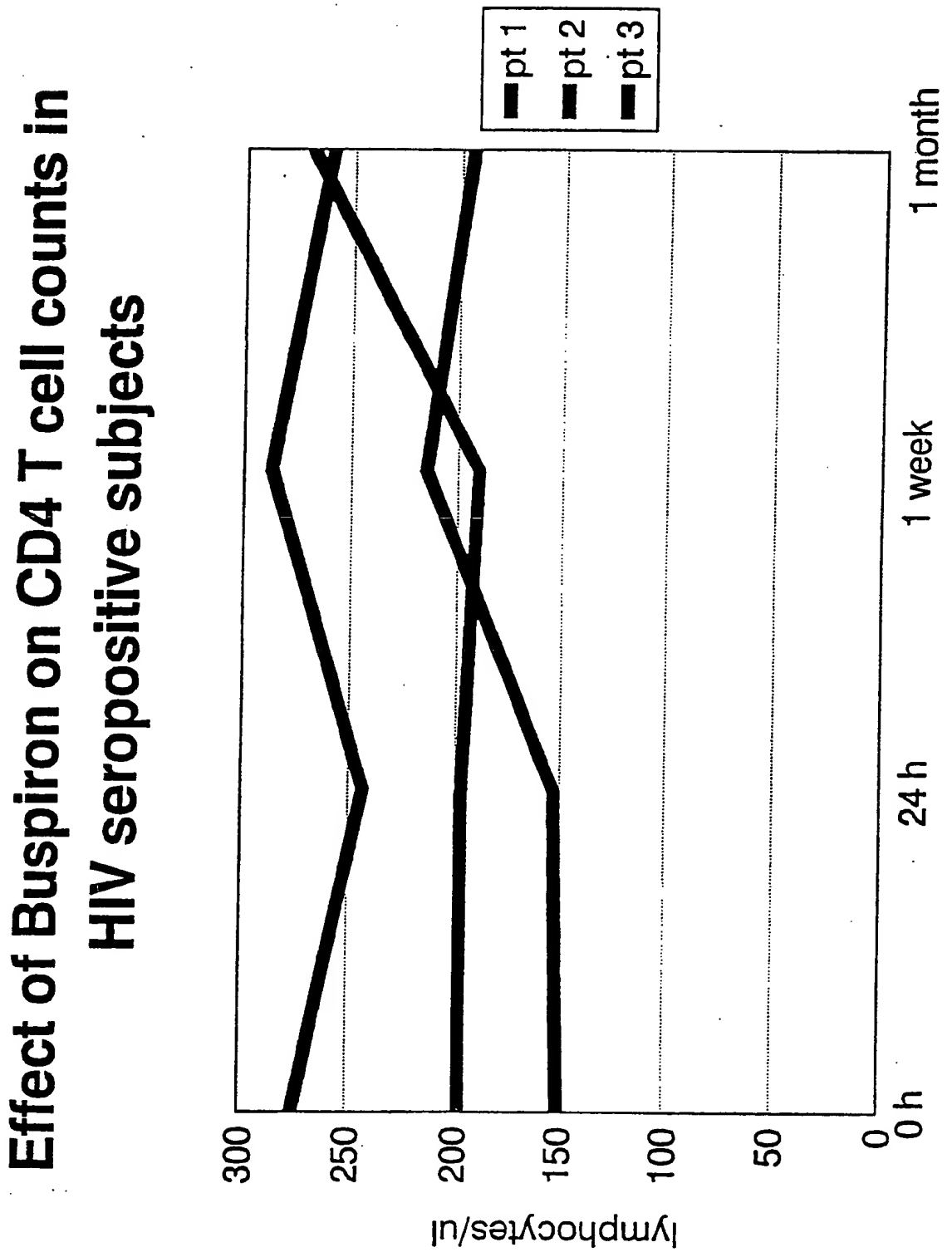
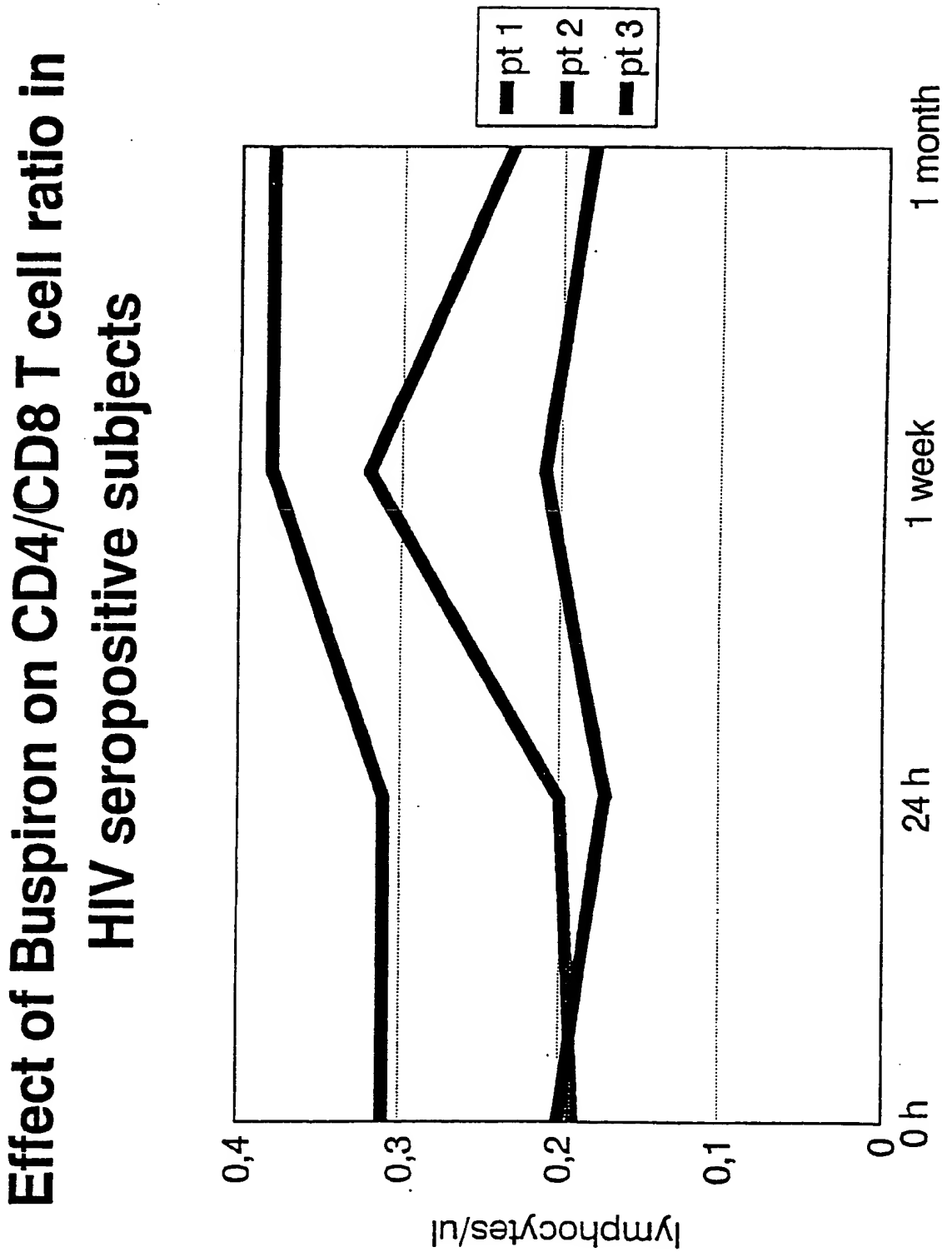

Effect of Buspiron on CD4 T cell counts in HIV seropositive subjects
pt 1
pt 2
pt 3
lymphocytes/ul
300
250
200
150
100
50
0
0 h
24 h
1 week
1 month

FIG. 10


Effect of Buspiron on CD4/CD8 T cell ratio in HIV seropositive subjects
pt 1
pt 2
pt 3
lymphocytes/ul
0,4
0,3
0,2
0,1
0
0 h
24 h
1 week
1 month

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00286

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/135, A61K 31/40, A61K 31/505
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9204015 A2 (MILES INC.), 19 March 1992 (19.03.92) --	1-19,50-53

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 1995	21 -10- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/08/95

International application No.
PCT/DK 95/00286

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A2- 9204015	19/03/92	AU-A- 8499891	30/03/92
		AU-A- 8848291	30/03/92
		CA-A- 2090688	05/03/92
		CA-A- 2090689	05/03/92
		EP-A- 0547172	23/06/93
		EP-A- 0555231	18/08/93
		JP-T- 6500775	27/01/94
		JP-T- 6503816	28/04/94
		WO-A,A,A 9204014	19/03/92

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00286

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20-50,54-64
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.